between conformational changes in single molecules and other effects caused by dye photophysics or multimers.
Single molecule FRET measurements were conducted on a series of immobilized double-stranded and G-quadruplex DNA molecules using a widefield microscope. Donor (Cy3/TMR) and acceptor (Cy5) molecules were both excited via an alternated laser excitation scheme. The double stranded DNA samples serve as molecular standards and G-quadruplex DNA structures, in this context, are interesting as they show large conformation diversity.

407-Pos Board B207
Twist/Writhe Partitioning in DNA Minicircles
Mehmet Sayar, Alkan Kabakcioglu, Baris Avsaroglu.
Here we present a systematic study of supercoil formation in DNA minicircles under varying linking number by using molecular dynamics simulations of a two-bead coarse-grained model. Our model is designed with the purpose of simulating long chains without sacrificing the characteristic structural properties of the DNA molecule, such as its helicity, backbone directionality and the presence of major and minor grooves. The model parameters are extracted directly from full-atomistic simulations of DNA oligomers via Boltzmann inversion, therefore our results can be interpreted as an extrapolation of those simulations to presently inaccessible chain lengths and simulation times. Using this model, we measure the twist/writhe partitioning in DNA minicircles, in particular its dependence on the chain length and excess linking number. We observe an asymmetric supercoiling transition consistent with experiments. Our results suggest that the fraction of the linking number absorbed as twist and writhe is nontrivially dependent on chain length and excess linking number. Beyond the supercoiling transition, chains of the order of one persistence length carry equal amounts of twist and writhe. For longer chains, an increasing fraction of the linking number is absorbed by the writhe.

408-Pos Board B208
Mechanical Analysis Methodology for DNA Minicircles Observed by Cryo-Em
Todd D. Lillian, Troy Lionberger, Davide Demurtas, Julien Dorier, Andrzej Stasiak, Edgar Meyhofer, Noel Perkins.
Recent cryo-electron microscopy (cryo-EM) images of DNA minicircles, about 100 basepairs in length, provide a new perspective on the mechanics of DNA. In essence, the resultant 3-dimensional reconstructions capture the mechanically deformed state of the double helix at an instant in time. Such deformations may include sites with high bending and/or torsional flexibility that could result from the tight bending required to cyclize, superhelical stress, and thermal fluctuations. Unfortunately, these reconstructions resolve only the DNA helical axis and provide no information about (i) how to register their known basepair sequence with the reconstruction and (ii) how torsional deformations are distributed along the length of the minicircle. In addition, the experimental procedures are complicated and consequently limit the the number of reconstructed minicircles to about 20. Our objective is to understand the mechanics of these DNA minicircles, and specifically, to develop a method capable of detecting the presence of kinks or torsional destabilizations in their cryo-EM reconstructions. To this end, we developed a modal analysis approach to describe the mechanics of DNA minicircles. In our method, we use the thermal modes of a homogeneous elastic rod representation for circular DNA to assign modal amplitudes to each reconstruction. The distribution of modal amplitudes for a population of minicircles provides a unique 'signature' dependent upon several variables, including superhelical density and the presence of kinks or torsional destabilizations. To test our method and predict these signatures, we developed a statistical mechanics model to simulate ensembles of DNA minicircles. This model can represent sequence dependence (elasticity/curvature) and prescribed kinks or torsional destabilizations. Our preliminary analysis suggests that the observed signatures are inconsistent with a homogeneous elastic rod and thereby implicate the role of sequence dependence, kinks or torsional instabilities.

409-Pos Board B209
Temperature Dependence of DNA Persistence Length
Stephanie Geggier, Alexander Kotlyar, Alexander Vologodskii.
We have determined the temperature dependence of DNA persistence length, a, using two different methods. The first approach was based on measuring the j-factors of short DNA fragments at various temperatures. Fitting the measured j-factors by the theoretical equation allowed us to obtain the values of a for temperatures between 5 and 42 C. The second approach was based on measuring the equilibrium distribution of the linking number between the strands of circular DNA at different temperatures. The major contribution into the distribution variance comes from the fluctuations of DNA writhe in the nicked circular molecules which are specified by the value of a. The computation-based analysis of the measured variances was used to obtain the values of a for temperatures up to 60°C. We found a good agreement between the results obtained by these two methods. Our data show that DNA persistence length strongly depends on temperature and accounting for this dependence is important in quantitative comparison between experimental results obtained at different temperatures.

410-Pos Board B210
Sequence Dependence of DNA Persistence Length
Stephanie Geggier, Alexander Vologodskii.
It is vital for many aspects of DNA-protein interaction to know how DNA bending rigidity depends on its sequence. In particular, this is important for understanding how nucleosomes position along DNA molecules. Although the problem has been discussed for decades, it remains unsolved. The main difficulty is that one has to measure the rigidity constants for DNA molecules of different sequences with very high accuracy. The only known method that provides the needed accuracy is based on cyclization of short DNA fragments. Here we used the method to find how DNA bending rigidity depends on its sequence. We addressed the problem using the dinucleotide approximation, in which particular values of rigidity constants are assigned to each of 10 distinct dinucleotide steps. We prepared DNA fragments, about 200 bp in length, with various quasi-periodic sequences, measured their cyclization efficiency, and fitted the data by a theoretical equation to obtain the bending rigidity constant (or persistence length). By combining the data for all fragments we were able to extract a full set of rigidity constants. To test the resulting set of constants we used it to design DNA sequences that should correspond to very low and very high values of a, prepared the corresponding fragments and determined their values of a experimentally. We obtained remarkably close agreement between the measured and calculated values of a, proving that we have found the correct solution of this long-standing problem. This result opens new opportunities to test different models of sequence specificity of DNA-protein interaction.

411-Pos Board B211
The Mean Looping Time of DNA
Assaf Amitai, Ivan Kupka, David Holcman.
In many gene expression systems, a protein located on the DNA can affect the expression of a gene far along the chain. It has been recognized that the DNA can form transient loops, bringing a specific region of the gene close to another. Thus, transcription can be activated when a transcription factor is positioned far away from its site. The frequency of bending is a characteristic time scale of the activation process.
The mean time for a DNA molecule to loop, bringing together two sites, is a fundamental factor that we studied. Various approximations have been used to model polymers. Interestingly, dsDNA has been found to be well described by the standard Rouse model, in which the polymer is described as a collection of bead monomers connected by harmonic springs. The Rouse model is relevant when the sites are at a distance considerably bigger than the DNA persistence length. When the distance between the sites is of several persistence lengths, the semi-flexible chain model is better suited to model the DNA dynamics. The polymer chain is subjected to random independent motion (Brownian motion). When the two monomers come closer than a certain distance, interaction takes place and the monomers connect. We assumed that the interaction rate is much faster than the encounter time, thus the process ends with the first encounter of the monomers. This allowed us to compute the asymptotic formula for the mean encounter time in the two models. We obtained precise estimates for this mean first encounter time in two and three dimensions. Brownian simulations confirm our formulas and we discuss consequences of our results for random gene activation in the nucleus.

412-Pos Board B212
The Effect of Monovalent Cations on the Thermal Stability of Small DNA Oligomers with Internal Loops
Nancy C. Stellwagen, Paul J. Barnard.
Although the effect of monovalent cations on the thermal stability of nucleic acid duplexes has been studied for many years, relatively little is known about cation effects on the stability of DNAs or RNAs with internal loops. We have therefore investigated the thermal stability of the DNA analog of the *let-7* microRNA::*lin-41* messenger RNA complex from *C. elegans*, which contains an asymmetric internal loop that kinks the helix backbone[1]. A DNA construct containing a symmetric internal loop at the same site was also studied, as well as a fully base-paired control. The melting temperatures of the two oligomers with internal loops are equal at low ionic strengths in solutions containing Na^+ ions. However, the melting temperature of the oligomer with the asymmetric loop is ~2°C higher in solutions with Na^+ ion concentrations greater than ~100 mM. The melting temperature of the oligomer with the asymmetric loop is also ~2°C higher than the oligomer with the symmetric loop when the solution contains K^+ ions. Binding studies indicate that both oligomers with internal loops, as well as the duplex control, bind Na^+ ions weakly, with an average

dissociation constant of 150 ± 10 mM. Hence, high concentrations of Na^+ ions appear to stabilize DNAs with asymmetric internal loops, most likely because of electrostatic screening of the closely spaced phosphate groups near the kink site. Surprisingly, none of the oligomers bind K^+ ions over the concentration range tested.

Supported in part by the Analytical and Surface Chemistry Program of the National Science Foundation.

[1] M. Cevec, C. Thibaudeau, J. Plavec (2008) Nucleic Acids. Res. 36, 2330-2337.

413-Pos Board B213

Unfolding and Targeting Thermodynamics of DNA Hairpins Containing Internal Loops

Iztok Prislan, Hui-Ting Lee, Cynthia Lee, **Luis A. Marky**.

In this work, we use a set of DNA hairpins as a model to mimic a common motif present in the secondary structures of mRNA, i.e., a stem-loop motif with an internal loop in the stem. Specifically, we used a combination of UV and differential scanning calorimetry (DSC) melting techniques to determine thermodynamic profiles for the unfolding of a set of hairpins with sequence: d (GCGC**T$_n$**GTAACT_5GTTAC**T$_n$**GCGC, where n = 1, 3 or 5,"T_5" is an end loop of five thymines. UV melting curves of each hairpin show monophasic transitions with T_Ms that are independent of strand concentration, confirming their intramolecular formation. Analysis of the DSC profiles indicates that the unfolding of each hairpin results from the typical compensation of a unfavorable enthalpy (breaking of base-pair stacks) and favorable entropy contributions (release of ions and water molecules). The increase in the size of the internal loop from 2 to 10 thymines yielded: a) lower T_Ms and lower enthalpy contributions, corresponding to free energy contributions of ~0.7 kcal/mol of thymine; b) lower heat capacity effects that correlated with the lower releases of structural water molecules; and c) higher releases of ions. Furthermore, we used isothermal titration calorimetry to investigate the thermodynamics for the reaction of each hairpin with their partially complementary strands. The overall results showed that all three targeting reactions yielded favorable free energy contributions and were enthalpy driven. This approach works because of the favorable heat contributions resulting from the formation of base-pair stacks involving the unpaired bases of the loops. Supported by Grant MCB-0315746 from the National Science Foundation.

414-Pos Board B214

Characterization of DNA and RNA Ion Atmospheres Using Multiple-Energy Asaxs

Suzette A. Pabit, Steve P. Meisburger, Li Li, Joshua M. Blose, Christopher D. Jones, Lois Pollack.

The number and spatial distribution of small positively-charged ions around highly negatively charged DNA or RNA contribute to the free energy of binding in vitro and in vivo. However, the majority of charge compensating ions around nucleic acids forms a diffuse counterion "cloud" that is not amenable to investigation by traditional methods that rely on rigid structural interactions. With 2 x-ray energies, one near and the other 100 eV away from the ion absorption edge, we have successfully used Anomalous Small-Angle X-ray Scattering (ASAXS) to compare and differentiate the ion spatial distribution around comparably sequenced short DNA and RNA helices. Here, we present further information gained when using multiple x-ray energies (up to 5) in an ASAXS experiment. We describe proper treatment of multiple-energy SAXS data including absolute SAXS intensity calibration and measurement of scattering factors from x-ray fluorescence. We discuss the strengths and limitations of this approach and derive useful parameters in depicting the nucleic acid ion atmosphere.

415-Pos Board B215

Improving Electrostatic Descriptions of Ions Around Double-Stranded DNA

Zhipeng Wang, Yong Huang, Li Li, Dennis G. Thomas, Lois Pollack, Alexey Onufriev, **Nathan A. Baker**.

Ions play an essential role in governing the structure and function of nucleic acids, due to the large negative charge associated with the nucleic acid backbone. The addition of even small numbers of multivalent, positively charged ions induces intra-strand attraction in DNA and thus efficiently packages the extended polymer into compact toroids. Anomalous small angle X-ray scattering (ASAXS) has emerged as a powerful technique to report the spatial distribution of ions associated to nucleic acids with unprecedented levels of detail and resolution. To determine more detailed information about these highly mobile ions relative to the underlying nucleic acid surface requires tight coordination with theoretical or computational tools. Presently, very few robust theoretical or computational tools exist for understanding ion-nucleic interactions. Current atomically-detailed computational approaches that represent the solvent environment explicitly, as discrete water molecules and ions, are prohibitively expensive for systematic studies of biologically relevant structures on time scales needed to fully understand these interactions. The alternative approach of "implicit solvent" models, that represent solvent implicitly as a continuum, could potentially overcome this difficulty. However, most "implicit solvent" models reduce computational effort at the expense of simplifying assumptions that preclude their application to highly charged systems in the presence of concentrated salt solutions or multivalent ions. We have been examining the ability of traditional and "size-modified" Poisson-Boltzmann models to predict the distribution of multivalent ions at low concentration around B-form DNA. These predictions are tested through direct comparison with ASAXS data on similar systems. The goal of this work is to examine the role of size-exclusion in the well-known failure of traditional Poisson-Boltzmann approaches for describing multivalent ions in highly charged systems. This work lays the foundation for a systematic approach to improve implicit solvent models for nucleic acid systems.

416-Pos Board B216

The Influence of Osmolytes on Electrostatic Interactions Among DNA Duplexes

Joshua M. Blose, Suzette A. Pabit, Steve P. Meisburger, Li Li, Christopher D. Jones, Lois Pollack.

Osmolytes, which function as a vital component of the cellular stress response, are small, chemically diverse, intracellular organic solutes. Protecting osmolytes enhance protein stability via preferential exclusion, where denaturation of the protein in the presence of the osmolyte is less favorable than in an aqueous environment. Thus, the correct ratios of protecting to non-protecting osmolytes and protecting osmolytes to ions are critical to maintain protein structure and protein-nucleic acid interactions. In contrast to the effects of osmolytes on protein stability, structure, and function, there is much less understood concerning the effects of osmolytes on nucleic acids. Although non-protecting osmolytes can destabilize both protein and nucleic acid structures, protecting osmolytes have different effects depending on the complexity of the nucleic acid structure. Furthermore, the influence of osmolytes on the ion atmosphere surrounding nucleic acids is not well understood. As a first step in quantifying the effects of osmolytes on nucleic acid electrostatics we used small angle x-ray scattering (SAXS) techniques to monitor 25-bp DNA duplexes and their interactions in the presence and absence of sucrose, a protecting osmolyte and important contrast matching agent in SAXS studies of protein-nucleic acid complexes. Results will be discussed.

417-Pos Board B217

Determination of the Composition of the Ion Atmosphere of Condensed DNA Utilizing Inductively-Couple Plasma Atomic Emission Spectroscopy

John Giannini, Xiangyun Qiu, Kurt Andresen.

The problem of reentrant DNA condensation has been studied for decades. Yet despite the immense amount of theoretical and experimental work on this problem, a definitive, experimentally verified model of condensation remains elusive. Using inductively-coupled plasma atomic emission spectroscopy, we have measured the ion composition of condensed DNA under a variety of solution conditions. We have studied not only the ion atmosphere in the condensed DNA, but also how the osmotic pressure and the presence of competing, non-condensing ions affect the final condensed ion atmosphere. These data provide a strong basis against which to measure the various theories of condensation.

418-Pos Board B218

Comparing Double-Strand DNA and RNA Condensation

Li Li, Suzette Pabit, Steve Meisburger, Lois Pollack.

DNA condensation is of great interest due to its fundamental biological importance. With the discovery of the important roles of RNAi, recent attention has been focused on efficient packaging of dsRNA for therapeutics. In this study, we applied UV spectroscopic and small angle x-ray scattering to investigate the mechanism of RNA condensation. Our results show that double-strand DNA and RNA behave very differently under certain ionic conditions. The forces that lead to side-by-side attraction and subsequent condensation of DNA molecules may be highly correlated with the differing geometric property of RNA and DNA.

419-Pos Board B219

Nucleic Acid Helical Conformation and Sequence Effects on Cationic Binding

Latsavongsakda Sethaphong, Yaroslava Yingling.

Conformation dependent molecular recognition has often been more associated with proteins which must be able to sense thousands of different molecules within a cell. Despite lacking as extensive a repertoire, nucleic acids also depend on nuances of structure with their environment to gain specificity for regulating genetic duplication, editing, expression, and suppression. In order to explore this topic further, molecular dynamics simulations of nucleic acid dupelexes of DNA and RNA were performed to examine subtleties with their inherent cation binding behavior. We discovered that despite small differences